SYNERGIC EFFECTS OF BASE EXCISION REPAIR AND OXIDIZED dNTPs POOL ON (CAG)n/$(CTG)_n$ REPEAT DYNAMIC INSTABILITY

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Several neurodegenerative diseases are caused by trinucleotide repeat (TNR) expansion . Although the mechanism is not fully understood, it has been proposed that TNR expansion can occur during long-patch base excision repair (BER) of the oxidized DNA base 8-oxo-7,8-dihydroguanine (8-oxoG). Recent studies in Huntington's disease (HD) indicate that $(CAG)_n/(CTG)_n$ repeat instability might also depend on the lack of coordination between DNA polymerase β (Polβ)-dependent repair synthesis and flap endonuclease 1 (FEN1) cleavage. This might generate non-B form DNA secondary structures (loop or hairpin) which are difficult to remove and might be integrated into the genome. It has been suggested that this is due to aberrant levels of these repair proteins in the brain of disease R6/1 HD mice.

To further investigate the molecular basis of $(CAG)_n/(CTG)_n$ expansion during BER of 8-oxoG, we chose an experimental strategy based on *in vitro* enzymatic reactions that reproduce the specific conditions of repair synthesis of damaged DNA. We used synthetic 100bp duplex oligonucleotides containing 20 CAG/CTG repeats and a single 8-oxoG located in the first triplet and purified enzymes and/or cell free extracts from different sources (mouse embryo fibroblasts and human cell lines, brain tissues). In addition levels of the BER proteins will be measured in different brain areas of another model for HD, the R6/2 mice.

We hypothesized that $(CAG)_n/(CTG)_n$ expansion might be favoured not only by direct oxidative damage to DNA, but also by incorporation of oxidized triphosphates during repair synthesis. For this purpose the *in vitro* repair assay was performed using a purified polβ and dNTPs supplemented with 8-oxodGTP. Our preliminary results confirm the role of long-patch BER in modulating TNR expansion. Moreover we were able to show that 8-oxodGTP is incorporated opposite either C or A during Polβ-dependent repair synthesis. As expected OGG1 DNA glycosylase was able to repair the newly formed 8-oxoG:C mismatches in TNR. We are still investigating whether the MUTYH DNA glycosylase can remove A from 8-oxoG:A mispairs in this sequence context. A complex scenario might be envisaged because of parallel processing of these mismatches on opposite strands. We propose that remodeling of repair products from subsequent repair events, closely related along the DNA sequence, will establish a 'toxic oxidation cycle' that favours $(CAG)_n/(CTG)_n$ expansion.